

FGIS Issuance Change

CHANGE TO**9** DIRECTIVE**9** MANUAL**X9** HANDBOOK

CHANGE NO: 1	TO (No.)	TITLE: Don (Vomitoxin) Handbook	DATE: 5-20-02
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PURPOSE OF CHANGE: The Don (Vomitoxin) Handbook has been revised to change the procedures for mixing substrate/chromagen and stop solutions in the RIDASCREEN®FAST DON test kit (Chapter 10), specify scale requirements for weighing sample portions (Chapter 3), and to eliminate the use of a filtering syringe in the extraction procedures for the AgriScreen, Veratox, and Veratox 5/5 Don test kits (Chapters 5 and 6).

FILING INSTRUCTIONS

Remove the obsolete pages and insert the revised pages as listed below.

Remove	Dated	Insert	Dated
Chapter 3	12-17-01	Chapter 3	5-20-02
Page 5-1	12-17-01	Page 5-1	5-20-02
Page 5-2	12-17-01	Page 5-2	no date
Page 6-1	12-17-01	Page 6-1	5-20-02
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Page 10-3	12-17-01	Page 10-3	5-20-02
Page 10-4	12-17-01	Page 10-4	no date

Retain this issuance sheet as an aid in verifying handbook contents.

/s/ David Orr

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DON HANDBOOK
CHAPTER 3
5-20-02

CHAPTER 3

SAMPLE PREPARATION

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3.1 SAMPLE SIZE AND PREPARATION

A sample of approximately 200 grams, with dockage and stones removed, is required for the DON testing and file sample (100 grams work portion, 100 grams file portion). An additional sample may be required if subsequent review inspections are requested. A similar sample size is recommended for submitted samples.

Obtain samples according to the guidelines in the Grain Inspection Handbook, Book I, "Grain Sampling." From the 100-gram work portion, divide (using a Boerner divider) out a portion of 50 grams for DON testing and weigh on an FGIS-approved type scale with a minimum division size of 0.1 gram.

3.2 GRINDING SAMPLES

Grind approximately 100 grams (dockage and stone free) of grain using a Romer Mill-Model 2a, Udy Grinder, Perten Falling Number Mill, Bunn Commercial Coffee Grinder, or an equivalent device that meets FGIS' performance requirements.

SAFETY NOTE: OPERATOR MUST OBSERVE SAFETY PRECAUTIONS AND WEAR EYE PROTECTION WHEN OPERATING THE GRINDER. SEE THE OPERATOR'S MANUAL FOR MORE SAFETY TIPS.

The grinding apparatus must be adjusted to produce a particle size that is sufficiently fine enough to obtain a homogeneous blend. Generally, a sufficiently coarsely ground sample of wheat resembles whole wheat flour, while a sample that is too coarsely ground has the appearance of bulgur or semolina. Avoid over-grinding or pulverizing a sample because it produces an excessively powdery mix that will slow down the filtration process.

a. Procedures for Checking the Performance of the Grinder.

To check the performance of equipment used for grinding **small grains (e.g., wheat and barley)**, use the following procedures:

- (1) Grind a sample portion of approximately 100 grams of relatively dry wheat (i.e., 13 percent or less moisture).
- (2) Weigh the entire portion that was ground.
- (3) Sieve the portion across a standard No. 20 wire woven sieve.
- (4) Weigh the portion that passed through the sieve.

- (5) Determine the percent of fine material, by weight, as follows:

Fines = weight from step (4) divided by the weight from step (2) X 100.

For locations that perform mycotoxin testing on coarse (e.g., corn) and small grains, perform the check using a 100-gram sample portion of corn having a moisture content of 14 percent or less.

b. Optimum Particle Size.

The optimum range for particles of coarse and small grain passing through the No. 20 sieve is between 60 and 75 percent. Whenever the ground particles appear to be too coarse, or the results of a grinder check indicate that less than 50 percent of the ground portion passes through the No. 20 sieve, the grinder should be adjusted or repaired to meet the optimum range requirements.

Grinding apparatuses must be checked periodically to determine whether they are producing a final product that meets the particle size requirements as listed above. Official personnel shall determine the frequency of the checks based on a number of items that include visual observation of the ground product, number of samples ground since last check, and time (number of days) since the last check was performed. Record all particle check results in a convenient location for future reference purposes.

5.1 TESTING AREA

The extraction solution and other materials used in the AgriScreen and Veratox test kits do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

5.2 EXTRACTION PROCEDURES

a. Barley, Corn, Oats, and Wheat.

- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
- (2) Label the collection container with the sample identification.
- (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
- (4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
- (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
- (6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.
- (7) Filter the extract by pouring at least 15 ml through the filter paper.

b. Malted Barley.

Follow step numbers 1-7 listed above, then pass 3 ml of the filtered extract through a Bond Elut SPE cartridge at a flow rate of 1 ml per minute.

5.3 PREPARATION OF SOLUTIONS

a. Conjugate.

- (1) Open one of the conjugate bottles and remove the rubber stopper.
- (2) Cut the tip off the enclosed squeeze tube and squeeze the tube contents into the bottle.
- (3) Replace the stopper and swirl contents until the pellet has dissolved.

ALLOW THE REHYDRATED CONJUGATE SOLUTION TO SET FOR 1 HOUR PRIOR TO USE.

Use the contents of the bottle until empty (**once rehydrated, contents must be used within 3 weeks**).

KEEP REFRIGERATED WHEN NOT IN USE.

b. Substrate.

Substrate is pre-activated, ready for use, and should be stored in the dark. Remove only one vial of substrate at a time from the foil pouch prior to use.

c. Stopping Reagents and DON Control.

Open the stopping reagents and the DON control bottles and set aside. Swirl to mix prior to use.

5.4 QUALITATIVE (SCREENING) TESTING

a. Testing Procedures.

NOTE: The AgriScreen kit is supplied with a 1 ppm control. Users must purchase another control to perform screening at a different level.

- (1) Remove the red-marked mixing well strip and break off the needed number of wells (one well for each sample and one well for control). Return the unused strip to the package.

6.1 TESTING AREA

The extraction solution and other materials used in the Veratox 5/5 test kit do not necessitate the use of separate FGIS-approved laboratory space. FGIS personnel may perform the testing in an FGIS-approved laboratory or in alternate testing space (i.e., table top in an inspection lab) upon approval of the field office manager. FGIS employees must comply with all applicable safety and sanitation requirements as listed in this handbook to ensure a safe and efficient work environment.

6.2 EXTRACTION PROCEDURES

- a. Wheat, Oats, Barley, Malted Barley and Corn Tested at the 5 ppm Conformance Limit.
 - (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
 - (2) Label the collection container with the sample identification.
 - (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
 - (4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
 - (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
 - (6) Let the extract sit for 2 minutes to enable some of the sample to settle before filtering the extract.
 - (7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.
 - (8) Dilute the sample extract 1:2 (1+1) with deionized or distilled water. (For example, add 1.0 ml of extract to 1.0 ml of deionized or distilled water.)
 - (9) Mix well.
 - (10) Proceed to test analysis steps.

b. Optional Procedures for Testing Barley and Malting Barley at the 2.5 ppm Conformance Limit.

- (1) Place a sheet of filter paper (Whatman #1 folded or S&S 24-cm pleated or equivalent) into a clean funnel mounted over a 25 x 200 mm (diameter x length) test tube or collection beaker.
- (2) Label the collection container with the sample identification.
- (3) Thoroughly mix the ground sample and weigh a 50-gram portion.
- (4) Place the ground 50-gram portion into an 18-ounce Nasco Whirlpack bag or similar type of sealable plastic bag.
- (5) Add 250 ml of distilled or deionized water and shake (by hand or mechanically) for 3 minutes.
- (6) Let material stand for 2 minutes to enable some of the sample to settle before filtering the extract.
- (7) Filter the extract by pouring through the filter paper into the labeled sample jar. Collect a minimum of 15 ml of the extract.
- (8) Proceed to the analysis steps.

6.3 TEST PROCEDURES

a. Analysis Procedure.

- (1) Allow reagents, antibody coated wells, mixing wells, and sample extracts to reach room temperature prior to running the test (approximately one hour).
- (2) Remove one red-marked mixing well for each sample to be tested, plus five red-marked wells to be used for controls. Place these wells in the microwell holder.

- (6) Using a pipettor, mix the wells by pipetting the liquid up and down in the tips 3-4 times.
- (7) Incubate for 5 minutes (\pm 1 minute) at room temperature.
- (8) Dump the contents of the wells. Turn the wells upside down and tap out on a paper towel until the remaining liquid has been removed.
- (9) Using a wash bottle, fill each well with washing buffer solution. Empty the wells again and remove all remaining liquid. Repeat this step 2 times (total of 3 washes).
- (10) Add 100 μ l of substrate/chromagen (white dropper bottle) to each well.
- (11) Mix thoroughly by gently sliding the plate back and forth on a flat surface.
- (12) Incubate for 3 minutes (\pm 0.5 minutes) at room temperature (64 – 86° F). Cover the wells with a paper towel to protect them from light sources.
- (13) Add 100 μ l of stop solution (yellow or orange dropper bottle) to each well.
- (14) Mix thoroughly by gently sliding the plate back and forth on a flat surface.
- (15) Measure absorbance at 450 nm using the EL 301 microwell reader (Results must be read within 10 minutes.)

b. Reading the Results.

- (1) Make sure that the microwell reader is on and allowed to warm up for a minimum of 15 minutes before using.
- (2) Remove sample carriage and hit "Enter."
- (3) Insert W2 filter and hit "Enter."
- (4) Insert W1 filter (450 nm) and hit "Enter."

- (5) Hit "Clear" and then "Blank." This will cause the instrument to read air as the blank sample.
- (6) Load antibody-coated wells into sample carriage so that the first control labeled 0 is in position A1.
- (7) Load the sample carriage into the strip reader so that position A1 is under the light beam of the reader.
- (8) Press "Read" and an absorbance value for A1 should appear in the display on the microwell reader. Record the value.
- (9) Slide the carriage to position A2 and press "Read." An absorbance value for A2 will appear. Record the value.
- (10) Repeat step (9) until absorbance values have been obtained for all controls and samples. Record the values.

c. Calculating the Results

Use the RIDA®SOFT Win Data software provided by r-Biopharm to convert the absorbance values into concentration values.

10.5 REPORTING AND CERTIFYING TEST RESULTS

Report all results on the pan ticket and inspection log to the tenth ppm unless the result exceeds 5.4 ppm. Results exceeding 5.4 ppm are reported as > 5.4 ppm unless a supplemental analysis is performed.

When test results indicate that DON is present at a level of 0.5 ppm or less, certify the results as "equal to or less than 0.5 ppm."

Test results between 0.6 ppm and 5.4 ppm are certified to the nearest whole ppm.

Test results over 5.4 ppm are certified as exceeding 5 ppm unless a supplemental analysis is performed.

Refer to the Certification section of the handbook for more detailed certification procedures.